Biomarkers for Toxicology Studies
September 20-21, 2006 at the NIEHS

Objectives. The NTP sponsored a workshop to identify additional biomarkers of lung disease, heart disease, and altered carbohydrate and lipid metabolism (e.g., metabolic syndrome) that could be assessed routinely in NTP subchronic toxicology studies to better characterize endpoints of environmentally-induced disease or biological process related to human disease etiology. The workshop organizers selected these areas of focus after surveying major human diseases and disorders with respect to mortality, incidence, economic impact, and relationship to environmental factors. The program included plenary sessions as well as three breakout group meetings for in-depth discussions. Additional information about the meeting, including background materials and presentations are available on the NTP website at http://ntp.niehs.nih.gov/go/20940. Findings from the workshop will be presented to the NTP Board of Scientific Counselors at its meeting on December 1, 2006. The following text summarizes major points of the workshop discussion.

Workshop Discussions

Lung:
The lung breakout group discussed a variety of potential approaches for collecting biomarkers ranging from lavage fluid analysis, respiratory function, enhanced tissue pathology, imaging, to gene analysis and proteomics. Of these, the group felt three would be most useful for the NTP: (1) bronchoalveolar lavage analysis (BAL), (2) enhanced histopathology, and (3) gene expression analysis. The group also believes imaging techniques hold great promise, but they are still in the late stages of refinement.

The group felt BAL would be especially appropriate for obtaining cell counts and differentials. There are a significant number of molecular biomarkers that can also be obtained through lavage analysis (e.g., chemokines, cytokines, antioxidants, albumin, etc.,) but the group did not make specific recommendations on which of these to include. Instead, they recommended the NTP select a panel of markers to evaluate processes such as immunity (innate and acquired) and inflammation. The group did not necessarily believe the biomarkers obtained from BAL would be useful to identify an "adverse" effect level.

Histopathology markers can address lung injury, inflammation, apoptosis, repair and other events either early or late in the disease process. Specifically, the group suggested the NTP include trichrome and Periodic Acid Schiff (PAS) stains and Ki67 protein for cell proliferation. In addition, conducting immunohistochemistry for proteins assessed in BAL would be useful for cross-platform confirmation purposes. Depending on the specific marker, such measures may also be useful to determine an adverse effect.

The group spent a significant amount of time discussing the use of gene expression data. However, the bioinformatics required to properly interpret the data are not as advanced as the technology which prohibits the use of gene expression data on a large scale. Also, to aid interpretation it is necessary to correlate genotypic and phenotypic changes. For these reasons, the group recommended the NTP explore the use of gene expression analysis not as a routine measure, but on a more limited basis.

Heart:

The heart breakout group recommended the routine inclusion of three biomarkers in NTP subchronic studies: troponin, α2-macroglobulin in the rat, and B-type natriuretic protein (BNP) in conjunction with ultrasound. All of these biomarkers are considered indicative of a disease process rather than predictive.

Troponin (TN) is an obvious candidate biomarker. Troponin is a component of the heart muscle and its release is indicative of early events in heart tissue degeneration, necrosis, and myocyte damage. In humans, troponins are the preferred markers for diagnosis of myocardial injury and increased cardiac troponin is defined as a measurement greater than the 99th percentile of an appropriate reference group. While sensitive and specific it probably can not yet be used to demonstrate an adverse effect because reference ranges in the rodent are not established. The group did not recommend a specific type of troponin (i.e., TnT or TnI).

B-type natriuretic protein (BNP) is a hormone released by the heart during ventricular stress. It was recommended as a biomarker to evaluate myocardial pressure and volume overload. BNP is considered a strong "negative predictor" since a low value means volume parameters are normal. An elevated value is somewhat less sensitive since it indicates that a problem will likely occur but does not predict when it may manifest. BNP is typically elevated when pathological findings are noted. The group did not know whether an abnormal BNP level would constitute an adverse response. Although BNP assays based on serum samples are available for humans, in rodents the assay requires RNA extraction from the heart, which limits measurements at necropsy. The group suggested that the NTP develop a serum assay for rodents.

α2-Macroglobulin in the rat, analogous to human C-reactive protein (CRP), was recommended even though this marker is not cardiac specific because the group felt it important to have an indication of systemic inflammation. α2-Macroglobulin was suggested as a way to address potential effects on the vasculature, albeit, in a very non-specific way. Like BNP, α2-macroglobulin is considered to be a "negative" predictor such that a normal value indicates the absence of systemic inflammation and absence of vascular injury. An elevated α2-macroglobulin would probably be associated with vascular injury, including inflammation although reference ranges need to be established.

Ultrasound was discussed because it can evaluate any early and late disease process that alters heart function. It could likely be used to identify an adverse effect. Ultrasound is noninvasive and requires a live animal. It can be high throughput (40 - 60 animals a day with the right equipment and a skilled operator). For suspected cardiotoxicants, the group recommended imaging studies, when the technology becomes available.

Lipid/Carbohydrate Metabolism:

The three highest priority biomarkers identified by the lipid/carbohydrate metabolism breakout group are serum cholesterol/triglycerides, insulin, and glutathione. Histological analysis to separate microvesicular and macrovesicular fatty changes in the liver was recommended on a routine basis in order to distinguish different pathological processes.

Although somewhat expensive to measure, insulin was recommended because it is considered a better marker of insulin resistance than glucose. This is especially true for NTP studies where the animals are not fasted, which reduces the ability to interpret glucose levels. The breakout group suggested that NTP consider measuring glucose bound to hemoglobin in red blood cells (hemoglobin A1c or HbA1C) or fructosamine because they are less sensitive to feeding status. Alternatively, the NTP could consider fasting animals for approximately 4 hours prior to sampling.

Glutathione (GSH), a marker of whole body oxidative stress, was recommended even though it is not specific for metabolic disorders. Other workshop attendants had a concern that GSH is problematic because it can be difficult to interpret and analyze.

The group also identified several other biomarkers that would be appropriate for routine inclusion. Sterol regulatory element binding proteins in the liver (if histochemical techniques can be developed) indicate early events in cholesterol (SREBP 2) and fatty acid (SREBP 1) synthesis. Body composition analysis using dual-energy x-ray absorptiometry (DXA or DEXA) to evaluate lean mass, fat mass and bone density or microCT to distinguish visceral and subcutaneous fat was recommended either for routine use on a subset of animals or in special studies. Like the heart breakout group, this group thought general measures of inflammation, such as TNF-alpha and IL-6 could be useful as a routine measurement or in special studies even though changes are not specific to perturbations in lipid and carbohydrate metabolism. Other biomarkers could be triggered, such as liver triglycerides.

Other Major Plenary Recommendations:

- Create a decision tree for when triggered endpoints will be evaluated.

- Plan on conducting a retrospective analysis to find out which implementations were useful. Similarly, consider eliminating endpoints currently included that are not particularly useful.

- Consider markers for inflammation.